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DEVELOPMENT OF IMAGING AGENTS FOR THE VISUALISATION OF CARTILAGE IN VIVO

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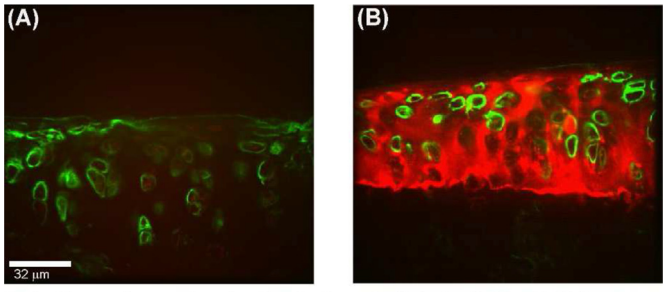
Purpose: OA disease progression in both humans and animal models is poorly defined by current X-Ray and MRI imaging methods. The main tissue of interest, cartilage, does not absorb X-rays and has non-optimal signal: noise with MRI, necessitating different MRI techniques. We aim to develop a cartilage-specific imaging agent to improve our ability to image OA disease progression in vivo.
Methods: We synthesised a family of imaging agents based on a DOTA (1,4,7,10-tetraazacyclododecane-1.4.7.10-tetraactic acid) backbone. DOTA offers the advantage of allowing multivalent decoration with a collagen II binding peptide for cartilage specificity, as well as being able to coordinate gadolinium ions for future MRI imaging. The Cy5.5 fluorophore was also incorporated to enable in vivo optical imaging and later visualisation of distribution by confocal fluorescence microscopy of cryosecions of the joint post-mortem.
Results: Using in vivo optical imaging, we characterised the retention of the DOTA-collagen II binding peptide compounds and their scrambled controls following intra-articular injection. DOTA with one collagen II binding peptide (1TP) was retained in the joint with a half life of 210 hours, compared to 12 hours for its scrambled control (1SP). This half life was increased to over 1000 hours with 3 collagen binding peptides (3TP). Confocal fluorescence microscopy of cryosections of the joint showed that 1TP and 3TP was present in the cartilage, but was excluded from the pericellular matrix of the chondrocytes.
Conclusions: We have demonstrated that the DOTA-collagen II binding peptide imaging agents bind specifically to cartilage in vivo and that the strength of this binding can be changed with valency. Further work is being carried out to utilise these cartilage imaging agents in different imaging modalities to obtain information about the status of cartilage in vivo.



Confocal images of cryosections of cartilage showing probe distribution (Cy5.5, red) with the proteoglycan perlecan as a marker for the pericellular matrix (Alexa-488-secondary antibody, green) in articular cartilage after 24 h of (A) 1SP and (B) 1TP

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PREVALENCE OF LUMBAR DISC DISEASE IN PATIENTS REFERRED FOR MRI IN GENERAL PRACTICE

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Purpose: The use of MRI as the initial imaging for low back pain has become more common in general practice. Limited information is available about the characteristics of these referred patients. The objective of this study is to describe the characteristics and MRI findings of low back pain patients referred for MRI in general practice, and to investigate whether baseline characteristics differ between patients with and without specific findings seen on MRI.
Methods: Cross-sectional cohort study. Patients (aged 18 years and over) referred by their general practitioner for MRI of the lumbar spine were recruited. The MRI radiology reports were scored regarding the presence of bulging, disc herniation, nerve root compression, spinal stenosis, and spondylolisthesis. Information on patients' characteristics and characteristics of the complaint were derived from the baseline measurement. Cross-sectional differences between patients with and without specific MRI findings were analyzed using a Mann-Whitney U-test or a Fisher's exact test.
Results: A total of 683 low back pain patients (53% male) were included, with a mean age of 49.9 (range 19-80 years). The mean back pain severity was 6.6 (SD 2.0) and 67% of the patients reported having chronic low back pain. No MRI findings were only reported in 6% of the patients. Patients with disc herniation with nerve root compression (69%) were younger, more often male, had a slightly higher BMI and reported more often acute back pain compared with those without. Also, they reported more often pain radiating in the leg below the knee, and more often neurological symptoms of the leg(s). They had more severe leg pain (mean 6.0) and a higher level of disability as measured with the RDQ (mean 14.0). Patients with spinal stenosis (13%) were more often male and were of higher age. Patients with spondylolisthesis (18%) reported more often pain radiating in the leg below the knee and were of higher age compared with those without.
Conclusions: In total, 69% of the MRI reports mentioned disc herniation with nerve root compression. These patients were younger, more often male, had a slighter higher BMI, reported more often acute back pain, reported more often pain radiating in the leg below the knee, and reported more often neurological symptoms of the leg, had more sever leg pain and a higher disability.

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QUANTITATIVE MICRO-CT ANALYSIS OF BONE IN ZEBRAFISH FOR STUDIES OF OA

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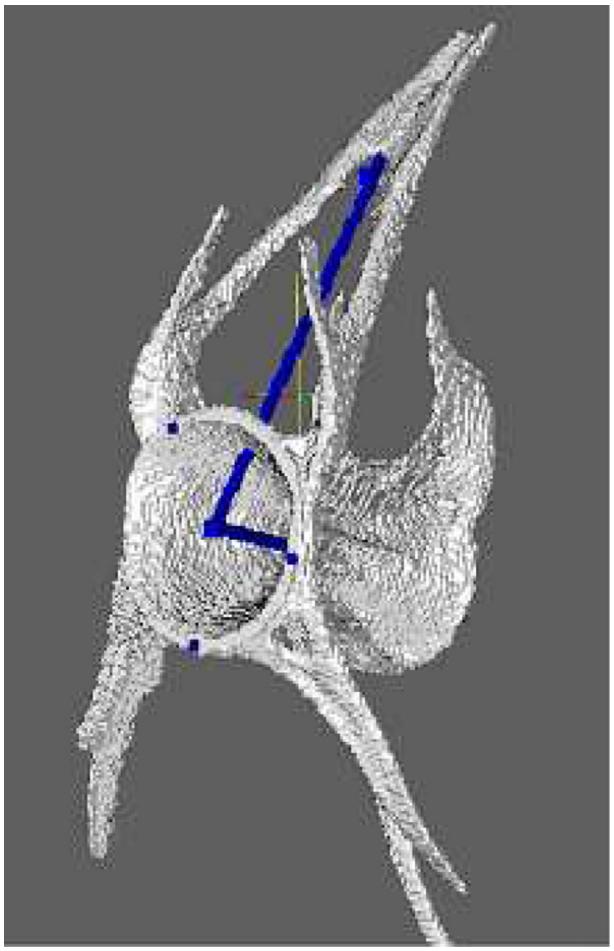
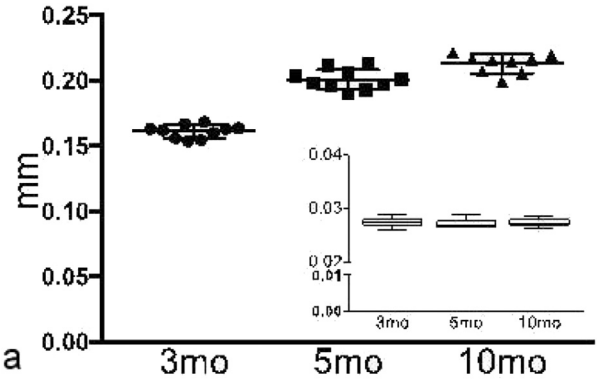
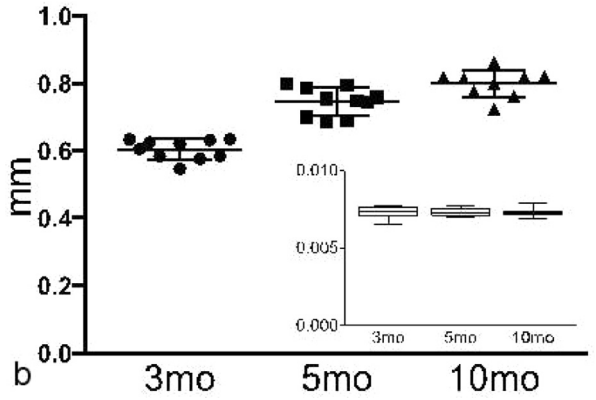
Purpose: Zebrafish are commonly used to investigate the genetics of disease incidence and progression. Recent studies have identified the zebrafish as a potential model for age related bone degeneration and remodeling given the similarities in bone formation and remodeling to higher vertebrates. However, a lack of analytic techniques to assess bone quality and shape has delayed the use of this model to study diseases of the bone and joint such as osteoarthritis (OA). The purpose of this study was to develop a rapid method to quantify the morphology and remodeling of zebrafish vertebrae.
Methods: Wildtype male sibling zebrafish (Dania rerio) strain AB were raised under standard conditions and fixed in formaldehyde at the indicated ages 3, 5, and 10 months. Sex and standard length were determined at the time of fixation. The fish were imaged at 6 micron isotropic resolution using a Scanco microCT-35 system. Using custom software, a reader placed landmarks easily identified on the CT images. From these points, the software segmented the vertebral body in 3D (Figure 1), and provided measurements of the caudal vertebral length, radius of vertebral opening, vertebral volume, and bone density. Statistical analysis was performed using GraphPad Prism version 6 and one way ANOVA or unpaired Students' t-test as indicated.
Results: Measurements of the first caudal vertebral body are reported. Systematic change in each measurement was observed in an age dependent manner (Fig 2). Quantitative analysis of first caudal vertebrae demonstrates differences in: length (2a), radius (2b), vertebral volume (2c), and BMD (2d). Note that the ratio of radius/length (standard length (2a and 2b insets) is consistent with isotropic growth; 1 way ANOVA $p<0.0001$ for all comparisons. Results for the precaudal and second caudal vertebrae were similar to the first caudal vertebrae. In zebrafish aged 3-10 months, there was no sex difference found across the parameters measured. Statistical analysis demonstrated significant age-related dependences of the length, radius, vertebral volume, and

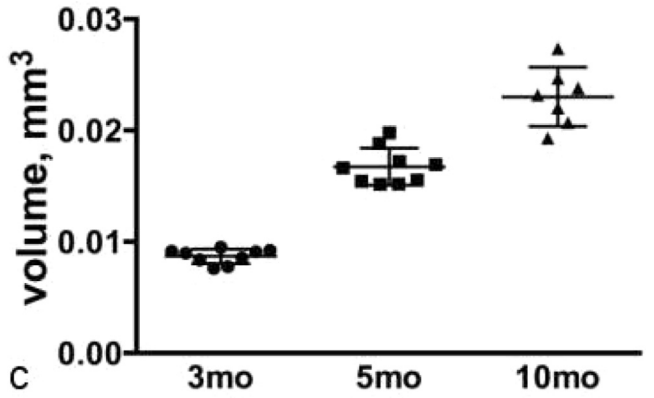
BMD. The measurement required less than 10 minutes of reader time per scan.

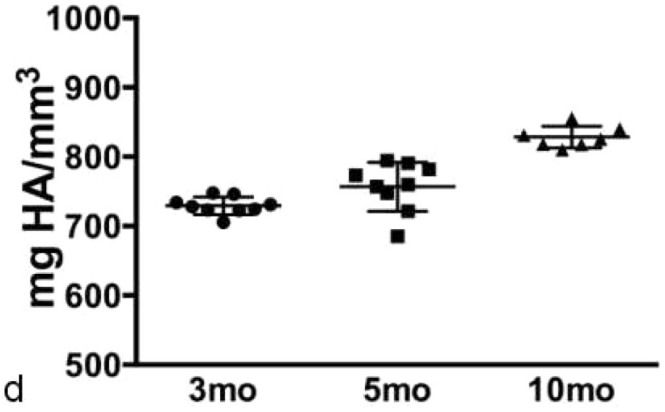
Conclusions: Recent studies have found that, in addition to providing a model of skeletal development, the zebrafish is a potentially powerful model of age related degenerative changes in the bone. Specifically, changes in the spinal structure of older fish were noted, which may provide an analogy of human disease. In order to measure these age related changes in zebrafish tissues, we have developed an efficient software tool that may be used for future studies of OA and other degenerative bone diseases. This method provides a step toward using the zebrafish as a model for human bone and joint disease such as OA.











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PHYSICAL INACTIVITY IS ASSOCIATED WITH REDUCED LUMBAR INTERVERTEBRAL DISC HEIGHT, HIGH FAT CONTENT OF PARASPINAL MUSCLES AND LOW BACK PAIN AND DISABILITY

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Purpose: Although physical inactivity has been associated with numerous chronic musculoskeletal complaints, few studies have examined its associations with spinal structures. Moreover, previously reported associations between physical activity and low back pain are conflicting. This study examined the associations between physical inactivity and structural abnormalities in the lumbosacral spine, as well as low back pain and disability.

Methods: 72 community-based volunteers not selected for low back pain underwent MRI of their lumbosacral spine (L1 to S1) in 2012.